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14. ABSTRACT: Dietary fat has been implicated as a potential promotional factor leading to progression of small, latent, non-metastatic prostate tumors to invasive, metastatic lesions. One possible mechanism is conversion of the n-6 polyunsaturated fatty acids to inflammatory compounds produced by the lipoxygenase (LOX) family of enzymes. We are examining whether genetic variants in the n-6 fatty acid LOX pathways are associated with the risk of prostate cancer in a population-based case control study of advanced prostate cancer among African-Americans and whites in Los Angeles County. In the first two years of the study, we genotyped five LOX gene polymorphisms, including 12-LOX Gln261Arg and Ser322Asn, 15-LOX-2 Gln656Arg, 5-LOX Lys254Glu, and the 5-LOX promoter Sp1 motif polymorphism. Preliminary analyses indicate that the 12-LOX gene Gln261Arg polymorphism may be related to prostate cancer risk in both African-Americans and whites. In the third year, we will investigate whether genetic variation in specific LOX pathways, in combination with diet, contributes to prostate cancer risk. Our findings could provide a scientific foundation upon which to design dietary intervention trials and may allow us to design strategies for reducing the disparity in prostate cancer burden between African-Americans and other ethnic groups.

15. SUBJECT TERMS

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Introduction:

Other than age, the strongest risk factor for prostate cancer is ethnicity and country of residence. African-Americans have higher mortality from prostate cancer than do other ethnic groups ("Cancer in California 1988-1997", California Cancer Registry, June 2000). It has been suggested that prostate cancer grows at a faster rate and exhibits more aggressive behavior in African-Americans (Powell and Meyskens, 2001). Dietary fat has been implicated as a potential promotional factor leading to progression of small, latent, non-metastatic prostate tumors to invasive, metastatic lesions (Snowden et al, 1984; West et al, 1991; Giovannucci et al, 1993). One mechanism by which fats might promote carcinogenesis is by conversion to eicosaoids, inflammatory compounds produced from n-6 polyunsaturated fatty acids by the lipoxygenase (LOX) family of enzymes (Steele et al, 1999). We hypothesize that dietary n-6 fatty acids, in combination with genetic variants in n-6 fatty acid LOX pathways may influence the development and progression of prostate cancer. Our specific aims are (1) to determine whether LOX genotypes are associated with risk of advanced prostate cancer in African-Americans and whites; (2) to determine whether LOX polymorphisms modify the effect of dietary fat intake on prostate cancer risk. We will test our hypotheses in a population-based case control study of advanced prostate cancer being conducted among African-Americans and whites in Los Angeles County. Using DNA samples for 860 cases (360 African-American and 500 whites) and 520 controls (230 African-American and 290 whites), we will genotype polymorphisms in lipoxygenase (LOX) family genes (5-LOX, 12-LOX and 15-LOXs). Logistic regression will be used to estimate odds ratios and test for effects of genotype and diet-genotype interaction. If we find that genetic variation in specific LOX pathways contributes to prostate cancer risk, this evidence will point to specific components of high fat diets that may increase risk. Such a finding will provide a scientific foundation upon which to design dietary intervention trials and may allow us to design strategies for reducing the disparity in prostate cancer burden between African-Americans and other ethnic groups.

Body:

In the approved Statement of Work, we proposed to finish the following work within the first 24 months funding (1 Dec 2003-30 Nov 2005):

a. DNA extraction and quantitation (Month 1-2);
b. Genotype 12-LOX gene Gln261Arg polymorphism (Month 3-6);
c. Genotype 12-LOX gene Ser322Asn polymorphism (Month 7-10);
d. Genotype 5-LOX gene promoter Sp1 motif polymorphism (Month 11-14);
e. Genotype 5-LOX gene Lys254Glu polymorphism (Month 15-18)
f. Genotype 15-LOX-2 gene Gln656Arg polymorphism (Month 19-22)
g. Clean up genotyping results (Month 23-24)

<u>To address task a:</u>

We have successfully extracted and quantitated DNA from 880 cases (378 African Americans and 502 whites) and 472 controls (163 African Americans and 309 whites).

<u>To address task b:</u>

We have successfully genotyped the 12-LOX gene Gln261Arg polymorphism on 1283 DNA samples. Minor allele frequencies were 32% among African-American controls and 42% among white controls.

Odds ratios for the association between genotype and prostate cancer risk were remarkably similar for the two ethnic groups (see table below). Compared to men carrying the GG genotype, men carrying AA appeared to have an approximate 22-23% (non-significant) reduction in risk. Men with the AG genotype were similar to the baseline group (GG).

African-Americans

	Controls	Cases	OR (95% CI)
GG	75 (46%)	172 (46%)	1.00
AG vs. GG	69 (43%)	169 (45%)	1.07 (0.72, 1.58)
AA vs. GG	18 (11%)	32 (9%)	0.78 (0.41, 1.47)
AA vs. GG+AG			0.75 (0.41, 1.38)
Total	162 (100%)	373 (100%)	

Whites

	Controls	Cases	OR (95% CI)
GG	113 (37%)	185 (37%)	1.00
AG vs. GG	132 (43%)	235 (47%)	1.09 (0.79, 1.49)
AA vs. GG	64 (21%)	81 (18%)	0.77 (0.52, 1.16)
AA vs. GG+AG			0.74 (0.51, 1.06)
Total	309 (100%)	501 (100%)	

For the two ethnic groups combined, the reduced risk associated with the AA genotype was statistically significant. Compared to men carrying the GG or GA genotypes, men carrying AA had an approximate 30% reduction in risk (see table below).

All men (African Americans & Whites)

	Controls	Cases	OR (95% CI)
GG	188 (40%)	357 (41%)	1.00
AG vs. GG	201 (43%)	404 (46%)	1.06 (0.83, 1.35)
AA vs. GG	82 (17%)	113 (13%)	0.73 (0.52, 1.01)
AA vs. GG+AG			0.70 (0.52, 0.96)
Total	471 (100%)	874 (100%)	

To address task c:

We have successfully genotyped the 12-LOX gene Ser322Asn polymorphism on 1287 DNA samples. Minor allele frequencies were 19% among African-American controls and 42% among white controls. The Ser322Asn polymorphism was in tight LD with the Gln261Arg in whites, hence among whites the odds ratios for Gln261arg were nearly identical to those for Ser322Asn. Among African-Americans, the two polymorphisms were not in tight LD. The Ser322Asn polymorphism was not associated with risk in African-Americans.

African-Americans

	Controls	Cases	OR (95% CI)
AA	108 (67%)	236 (63%)	1.00
AG	47 (29%)	123 (33%)	1.20 (0.80, 1.80)
GG	7 (4%)	14 (4%)	0.92 (0.36, 2.33)
Total	162 (100%)	373 (100%)	

Whites

	Controls	Cases	OR (95% CI)
AA	113 (37%)	189 (38%)	1.00
AG	133 (43%)	231 (46%)	1.04 (0.76, 1.42)
GG	63 (20%)	81 (16%)	0.77 (0.51, 1.15)
Total	309 (100%)	501 (100%)	

To address task d:
We have successfully genotyped the 5-LOX gene Sp1 motif polymorphism on 1334 DNA samples. The genotypes are summarized in the following table.

	African-American		Whites	
	Controls	Cases	Controls	Cases
2 / 4	1 (1%)	0 (0%)	0 (0%)	0 (0%)
2 / 5	0 (0%)	0 (0%)	1 (0%)	0 (0%)
3 / 3	15 (9%)	37 (10%)	0 (0%)	0 (0%)
3 / 4	10 (6%)	32 (9%)	0 (0%)	0 (0%)
3 / 5	42 (26%)	109 (30%)	2 (1%)	4 (1%)
3 / 6	3 (2%)	8 (2%)	0 (0%)	0 (0%)
3 / 7	0 (0%)	1 (0%)	0 (0%)	0 (0%)
4 / 4	9 (6%)	9 (2%)	7 (2%)	11 (2%)
4 / 5	31 (19%)	67 (18%)	81 (26%)	132 (27%)
4 / 6	2 (1%)	5 (1%)	1 (0%)	0 (0%)
4 / 7	0 (0%)	1 (0%)	0 (0%)	0 (0%)
5 / 5 (wildtype)	44 (28%)	85 (23%)	207 (67%)	337 (68%)
5 / 6	3 (2%)	8 (2%)	8 (3%)	11 (2%)
5 / 7	0 (0%)	7 (2%)	0 (0%)	3 (1%)
Total	160 (100%)	369 (100%)	307 (100%)	498 (100%)

To address task e:
We have successfully genotyped the 5-LOX Lys254Glu polymorphism on 535 African-American samples. This polymorphism was not genotyped in whites since it is rare in subjects of non-African ancestry. This polymorphism was not associated with prostate cancer risk.

African-Americans

	Controls	Cases	OR (95% CI)
GG	136 (84%)	308 (83%)	1.00
AG vs. GG	26 (16%)	63 (17%)	1.07 (0.65, 1.76)
AA vs. GG	0 (0%)	2 (1%)	
AA vs. GG+AG			1.10 (0.67, 1.82)
Total	162 (100%)	373 (100%)	

To address task f: We have successfully genotyped the 15-LOX-2 gene Gln656Arg polymorphism on 1343 samples. This polymorphism was not significantly associated with prostate cancer risk in African-Americans or whites.

African-Americans

	Controls	Cases	OR (95% CI)
CC	102 (63%)	233 (63%)	1.00
CT	49 (30%)	125 (34%)	1.12 (0.75, 1.67)
TT	11 (7%)	14 (4%)	0.56 (0.24, 1.27)
Total	162 (100%)	372 (100%)	

Whites

	Controls	Cases	OR (95% CI)
CC	86 (28%)	130 (26%)	1.00
CT	148 (48%)	243 (49%)	1.09 (0.77, 1.53)
TT	74 (24%)	128 (26%)	1.14 (0.77, 1.70)
Total	308 (100%)	501 (100%)	

To address task g: We have repeated all genotype assays that failed to amplify or that produced ambiguous results. We now have less than 1.4% missing data for all genotypes. Replicate samples showed 100% concordance.

Key Research Accomplishments

Successfully extracted and quantitated 1352 DNA samples;
Successfully genotyped the 12-LOX gene Gln261Arg polymorphism on 1345 DNA samples;
Successfully genotyped the 12-LOX gene Ser322Asn polymorphism on 1345 DNA samples;
Successfully genotyped the 5-LOX gene promoter Sp1 motif polymorphism on 1334 DNA samples.
Successfully genotyped the 5-LOX Lys254Glu polymorphism on 535 African-American samples.
We have successfully genotyped the 15-LOX-2 gene Gln656Arg polymorphism on 1343 samples.
Preliminary analyses indicate that the 12-LOX gene Gln261Arg polymorphism may be related to prostate cancer risk in both African-Americans and whites.

Reportable Outcomes:

None to date. (Pending final analyses)

Conclusions:

None to date. (Pending final analyses)

References

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Appendices

None